

Bacterial Retention during Filtration of a Live Attenuated Virus Vaccine through the Sartobran P Sterile Filter

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Abstract:

Recent studies of sterile filtration of a Live Attenuated Virus (LAV) demonstrated that the Sartobran P sterile filter provided 80% yield of an LAV that was 100 – 400 nm in size, raising questions about the effectiveness of this filter in retaining the standard challenge bacterium, *Brevundimonas diminuta*. This study evaluated the retention of *B. diminuta* by the Sartobran P over a range of conditions appropriate for LAV filtration. The *B. diminuta* were characterized by dynamic light scattering (DLS), nanoparticle tracking analysis (NTA), and scanning electron microscopy. The Sartobran P showed complete retention of *B. diminuta* under all conditions, even in the presence of additives like sucrose, surfactants, and high salt that have previously been hypothesized to increase the risk of bacterial breakthrough. The size of *B. diminuta* decreased when incubated in the nutrient poor media required by the ASTM challenge test. The addition of sucrose caused a further reduction in size as measured by NTA, although this was due to an increase in cell motility. There was no evidence of bacterial breakthrough at high loadings of either the LAV or *B. diminuta*, further demonstrating the effectiveness of the Sartobran P for sterile filtration of large viral vaccines.

Keywords: Vaccine, Viral vector(s), Unit operations, Filling, Formulation

1.0 Introduction

Sterile filtration is used to insure the sterility of nearly all liquid drug formulations. The early sterile filters were able to effectively remove yeast, molds, and most bacteria from both pharmaceutical and food products^{1,2}. However, in the 1960's, Bowman et al.³ identified a small gram negative bacterium, *Pseudomonas diminuta* (now referred to as *Brevundimonas diminuta*), that was able to pass through the pores of the sterilizing grade (0.45 µm pore size) filters that were widely used at that time. This led to the introduction of smaller 0.2 / 0.22 µm pore size membranes that could effectively retain *B. diminuta*. Sterile filters are currently qualified using standard protocol ASTM F838-83⁴, which requires a completely sterile filtrate after challenging the membrane with 10⁷ colony forming units (CFU) / cm² of *B. diminuta* grown under well-defined conditions.

Despite the extraordinary success of sterile filtration, a small number of studies have identified cases in which bacteria were observed in permeate samples collected through previously qualified sterile filters. For example, Lee et al.⁵ showed that certain growth conditions, e.g., use of saline lactose broth at low agitation rates, reduced the size of *B. diminuta* leading to penetration through 0.2 µm cellulose acetate filters. Howard and Duberstein⁶ reported similar results with microorganisms commonly found in water systems but noted that penetration was both time dependent and highly influenced by pH, liquid surface tension, and ionic strength. Folmsbee⁷ performed a retrospective analysis of more than 1000 filter validation failures and found that high bacteria load and high load rate were both correlated with the probability of bacteria breakthrough. Other studies have hypothesized that bacteria can deform to pass through the pores of sterile filters, particularly at high transmembrane pressures.⁸⁻¹¹ Helling et al.¹² found a strong correlation between pathogen / particle stiffness (evaluated using atomic force microscopy) and breakthrough

using polyethersulfone membranes. There is also some evidence that bacteria can grow in the filter matrix, with smaller daughter cells eventually migrating all the way through the sterile filter.^{6,13}

There are a number of unique challenges in applying sterile filtration for the production of vaccines and virus-like particles. First, many vaccines are similar in size to the 0.2 μm -rated pores of sterilizing grade filters, leading to substantial yield loss and high levels of membrane fouling. Vaccines often contain adjuvants to increase the immune response generated by the vaccine (including monophosphoryl lipid) and surfactants to reduce aggregation of vaccine particles. Folmsbee⁷ observed that 92% of failed challenge tests in their retrospective study were for formulations that were characterized as low surface tension ($<68 \text{ dyne/cm}^2$) due to the presence of these adjuvants or surfactants. Formulations with surface tension close to that of water (70 dyne/cm^2) showed a much lower risk of bacteria breakthrough.

Recent work from our laboratory has shown that the Sartobran P, a dual-layer cellulose acetate sterile filter, has excellent performance during sterile filtration of both a live attenuated viral vaccine (LAV) and a model nanoparticle suspension (NP).^{14,15} In particular, the overall LAV / NP yields were around 80% even though both suspensions had a mean particle size around 240 nm with some particles being greater than 400 nm in size (as determined by dynamic light scattering and nanoparticle tracking analysis). However, none of these studies examined the bacterial retention properties of the Sartobran P, raising questions as to whether the high LAV / NP transmission could potentially signal that this sterile filter might not provide complete retention of very small bacteria like *B. diminuta*. The objective of this study was to directly measure *B. diminuta* retention by the Sartobran P, including experiments under conditions that were previously identified as creating high risk for bacterial breakthrough. The results clearly

demonstrate the effectiveness of the Sartobran P sterile filter, with complete bacterial retention observed in all experiments.

2. Methods and Materials

Experiments were performed with *Brevundimonas diminuta* (*B. diminuta*) purchased from ATCC (Catalog number: 19146) as 6 frozen glycerol stocks and stored at -80°C until use.

A live attenuated viral vaccine (LAV) was acquired from Merck & Co. in a 25 mM histidine buffer with 75 mM NaCl and 9% sucrose; additional details on the LAV are provided elsewhere.¹⁶

200 and 300 nm fluorescently labeled polystyrene latex nanoparticles (NP) were purchased from Magsphere, Inc. (Pasadena, CA) at a stock concentration of 2.5 wt%. These particles were formulated as an 80:20 mixture in the presence of 0.01% Tween 20; the resulting suspension has been shown to be an appropriate model for the LAV in terms of its sterile filtration behavior.¹⁴

2.1 Media and buffers

Preparation of the *B. diminuta* involved three growth media: tryptic soy broth (TSB), lactose broth (LB), and saline lactose broth (SLB) as per ASTM F838-83. TSB was made by mixing 14 g of tryptone (VWR), 3 g of pancreatic digest of soybean meal (Acumedia), 5 g of NaCl (Sigma Aldrich), 2.5 g of dextrose (MilliporeSigma), and 2.5 g of sodium phosphate (MilliporeSigma) in 1 L of deionized (DI) water. TSB agar plates were made using the same formulation but with the addition of 8 g of agarose (MilliporeSigma). LB powder was purchased directly from Neogen (DOT Scientific); LB was made by mixing 13 g of powder with 1 L of DI water. SLB was made by mixing 30 mL of LB and 7.6 g of NaCl in 0.97 L of DI water. All media were autoclaved at 121°C for at least 15 min prior to use.

Phosphate buffered saline (PBS) was purchased from ThermoFisher as a 10x concentrate and diluted with DI water to achieve a final concentration of 10 mM phosphate at pH 7.8. Data were also obtained in the presence of different additives commonly found in vaccine formulations. Nonionic surfactants (Tween 20, Triton X-100, and Poloxamer 188) were purchased from MilliporeSigma. Tween 20 and Triton X-100 were diluted with DI water to make a 10 wt% stock solution, while the Poloxamer 188 was purchased at this concentration. Sucrose and NaCl were purchased from Omnipur and MilliporeSigma, respectively.

2.2 *Brevundimonas diminuta*

A working cell line was established by inoculating 50 mL of TSB with a single cryostock of *B. diminuta* in a 250 mL shake flask. The culture was grown in a VWR orbital shaking incubator at 30 °C and 150 rpm until attaining an optical density (OD) of 0.6. The cells were then transferred to a 50 mL centrifuge tube, pelleted at 2000 rpm using a Beckman Coulter Allegra A-12R centrifuge, resuspended in 10 mL of TSB containing 20% glycerol, and transferred to ten 1 mL cryovials for long term storage at -80 °C.

The purity of the working cell line was verified by inoculating a separate 50 mL of TSB with one vial of the working cell line by partially scraping the top surface of the frozen stock with an inoculation loop. The culture was grown at 30 °C and 150 rpm for 24 hr. A small sample was taken and diluted serially out to 1:10⁸ using 1% peptone in water. 100 µL of the final dilution was spread on an 8% TSB agar petri dish using a sterilized L-shaped spreader and incubated for 48 hr at 30 °C. The plate showed yellow-beige, shiny, and slightly convex colonies with no observable contamination, consistent with expectations for *B. diminuta*.

The suspensions used for the bacterial challenge were prepared by inoculating 50 mL of TSB with a small scrape sample of the frozen working stock in a 250 mL shake flask. The culture was incubated for 24 hr at 30 °C at a shaking rate of 150 rpm until the bacteria achieved a final OD of approximately 1.3 at 600 nm as measured by a Tecan Microplate reader. Approximately 1 mL of the TSB culture was transferred to 50 mL of SLB in a separate 250 mL shake flask using a serological pipette. The SLB culture was incubated for 24 hr at 30 °C and a shaking rate of 150 rpm, causing a reduction in the effective size of the *B. diminuta* (discussed subsequently). Approximately 1.6 mL of this culture was added to 50 mL of sterile PBS to create a bacterial challenge with a concentration of $\approx 4 \times 10^6$ CFU/mL. Additional additives such as surfactant and sucrose were added to the PBS and sterile filtered through a 0.2 μ m PES syringe filter (VWR) prior to addition of the bacteria.

B. diminuta concentrations were determined by serial dilution; feed samples were diluted approximately 10,000-fold with 1% peptone water and plated onto 8% TSB agar plates. Permeate samples were plated directly without dilution unless breakthrough was expected. All samples were analyzed in triplicate with the concentration of bacteria determined by the number of colonies.

2.3 Bacteria Challenge

The detailed procedures for the bacteria challenge were taken directly from ASTM F838-83. Data were obtained with the Sartobran P dual layered (0.45 / 0.2 μ m) cellulose acetate sterile filter (Sartorius) in both pre-sealed capsules and as small disks (25 mm diameter), with the latter placed in a stainless-steel filter holder (Pall). Filtration was performed at a constant filtrate flux of 300 L/m²/hr (LMH) using a Masterflex peristaltic pump, transferring the bacterial feed contained within a constantly stirred, sterilized reservoir (100 mL beaker) to the filter. The pump was

connected to an Ashcroft pressure gauge and then the capsule or stainless steel holder with the permeate left open to the atmosphere.

Immediately prior to the bacterial challenge tests, the filter capsule (or stainless-steel holder) was autoclaved for 15 min at 121 °C. The flow path was sterilized by pumping cold sterilant (Minncare) from the feed reservoir through the pump, tubing, and pressure gauge (without the filter). The lines were then flushed with sterile PBS prior to connecting the filter. Filtration experiments were conducted inside a Labconco laminar flow biohood using proper aseptic techniques to prevent bacterial contamination.

The hydraulic permeability of the membrane was evaluated by measuring the transmembrane pressure at several values of the filtrate flux to ensure that the membrane was not damaged. A permeate sample was then collected as a negative control to validate that the system was sterile. 1.6 mL of the *B. diminuta* were added to the feed reservoir containing 50 mL of sterilized PBS just prior to the challenge test to minimize any loss of cell viability. Permeate samples were collected every 25 L/m² in 12 mL aliquots for offline determination of the *B. diminuta* concentration.

2.4 Bacteria / particle characterization

The size distribution of the *B. diminuta* and LAV were examined by dynamic light scattering (DLS), nanoparticle tracking analysis (NTA), and scanning electron microscopy (SEM). DLS measurements were obtained with a Malvern Zetasizer Nano ZS using 70 µL samples run in triplicate. NTA measurements were performed using a Particle Metrix ZetaView®. Samples were diluted 10-fold using PBS to achieve approximately 200 particles in the viewing window. Analysis was performed at a frame rate of 3.75, a sensitivity of 65, and a shutter value of 200.

SEM images were obtained by first depositing the *B. diminuta* and / or LAV on a 0.1 μm Isopore membrane (MilliporeSigma) contained within a 25 mm stainless steel holder using a 5 mL sterile syringe. The membrane was removed from the holder, placed in a petri dish, and fixed using 2.5% glutaraldehyde for 30 min. The fixative solution was then discarded, and the membrane was washed for 15 min using PBS followed by a series of ethanol baths at 25, 50, 70, 85, 95, 100%, each for 5 min, to dehydrate the bacteria / LAV. The membrane was then dried using a Leica EM CPD300 critical point dryer to remove the ethanol. Samples were cut into 0.5 x 0.5 cm squares, placed onto an aluminum stud, and sputter-coated with a thin layer of gold/platinum using a Baltec SCD 050. Images were obtained with a Zeiss SIGMA VP-FESEM at 3.0 kV.

3. Results and Discussion

Figure 1 shows SEM images of *B. diminuta* deposited on 0.1 μm pore size Isopore membranes after growth in TSB media (top panel) and after being transferred to the nutrient-poor SLB media (bottom panel). The bacteria appear rod-shaped, mostly lying flat on the surface of the membrane, with a small number of bacteria oriented with the long diameter perpendicular to the surface (“standing up”). Switching to the nutrient poor media caused a reduction in the overall size of *B. diminuta*, consistent with results from previous studies.^{4,17} Based on the SEM images, the length was reduced from 1.2 to 0.6 μm while the width decreased from 0.4 to 0.3 μm , with the bacteria becoming somewhat more spherical in shape.

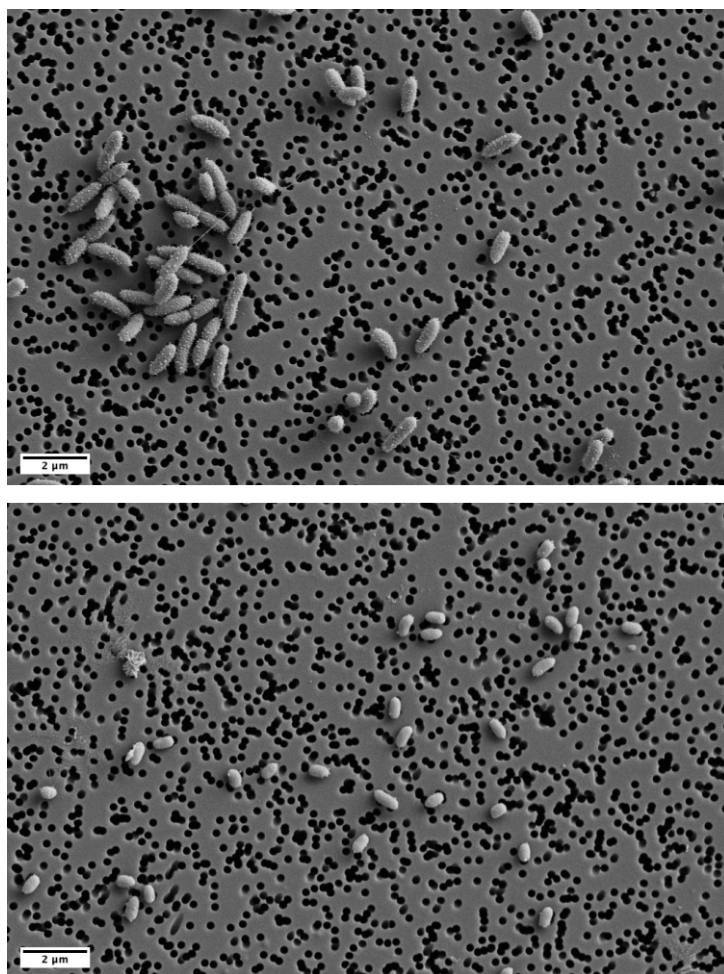


Figure 1: SEM images of *B. diminuta* in TSB (top) and SLB (bottom) deposited on 0.1 µm pore size Isopore membranes. The mean width and length of the bacteria grown in the TSB and SLB media were 0.4 x 1.2 µm and 0.3 x 0.6 µm, respectively.

Additional insights into the size of the *B. diminuta* were obtained by DLS, with the number distributions shown in Figure 2. The DLS results showed a shift in mean particle size from 850 to 690 nm when switching from the TSB to SLB media; this shift was consistent with the reduction seen in Figure 1 and with separate measurements performed using NTA.

Previous studies have shown that some live bacteria can have unusually large diffusion coefficients (compared to that based on their effective size) due to the motility of the bacteria.¹⁸

This was examined by treating the *B. diminuta* samples with formalin (37% formaldehyde) to kill the bacteria; the effectiveness of this treatment was confirmed by plating 100 μ L of the formalin-treated *B. diminuta* on a TSB agar plate with no colonies observed after 48 hr of incubation. The mean size of the formalin-treated *B. diminuta* was approximately 950 nm for the bacteria grown in TSB (100 nm larger than that determined for the live bacteria), but there was no observable difference in size for the *B. diminuta* in the SLB media with and without the formalin. These effects are consistent with the reduction in motility of the “starved” *B. diminuta* in the low nutrient SLB media.

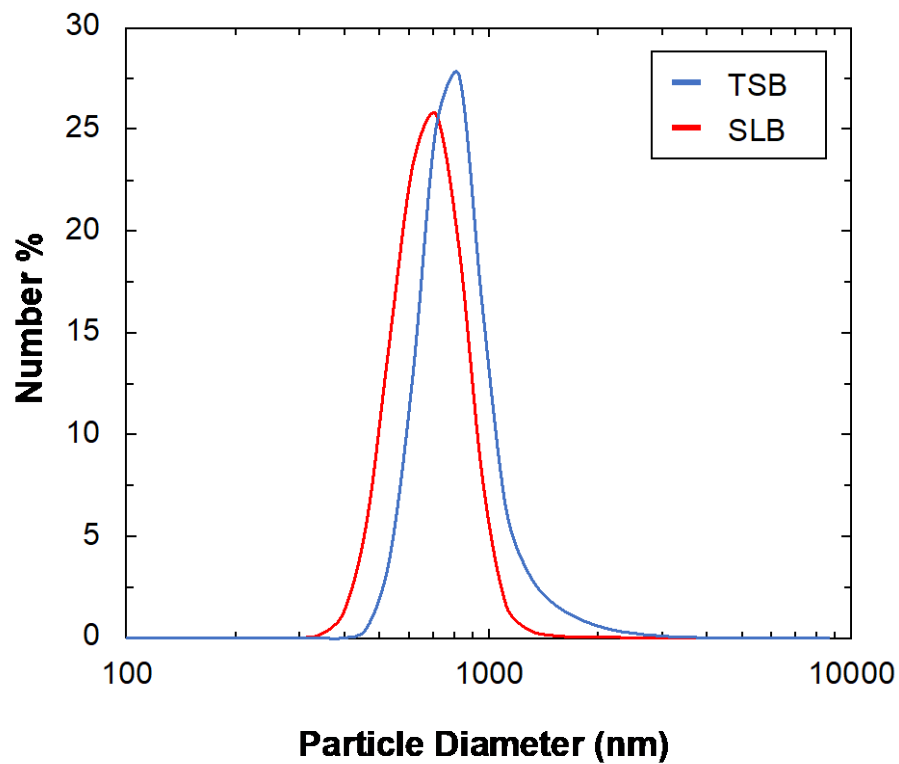


Figure 2: Particle size distributions of the *B. diminuta* determined by DLS in both TSB and SLB growth media (without treatment by formalin).

Initial bacterial challenge experiments were designed to validate our experimental system and to determine if either the challenge or growth conditions had any effect on bacterial retention. Several repeat experiments using the standard challenge conditions (*B. diminuta* grown in TSB and then incubated for 24 hr in the low nutrient SLB media) showed no viable colonies in any permeate samples. A control experiment using a 0.45 μm pore size cellulose acetate membrane, performed under the same conditions as the sterile filtration experiments (10 mM PBS at pH 7.8), showed significant bacterial breakthrough, with the bacteria concentration equating to approximately 10% of that in the feed, demonstrating that the *B. diminuta* were viable and able to pass through larger membrane pores.¹⁷ In addition, repeated circulation of the *B. diminuta* through the Masterflex pump and tubing caused no measurable change in viability. Limited experiments were performed with different agitation rates during incubation and different holding times before the filtration, neither of which had any effect on the measured bacterial size or retention. Note that increasing the agitation rate from 50 to 200 rpm did increase the final titer (from 8×10^8 to 2×10^9 CFU / mL) due to the improved oxygen mass transfer. Experiments performed at even higher bacterial challenge (1.6×10^{10} CFU/cm²) also showed no *B. diminuta* breakthrough. Note that the higher challenge experiment also used a higher feed concentration, leading to an increase in the transmembrane pressure from 3 kPa to more than 130 kPa after 700 L/m² of filtration; permeate samples obtained at this high pressure still showed no colonies of *B. diminuta*.

Table 1: Results from bacterial challenge experiments using different feed conditions along with the mean size of the live and formalin-treated bacteria determined by NTA.

Experimental conditions	Challenge (CFU/cm ²)	Test result (+/-)	Bacteria Size (nm)	
			Live	Formalin-killed
10 mM PBS (Standard challenge)	4.7×10^7	-	640	690
10 mM PBS (Moderate challenge)	1.9×10^9	-	-	-
10 mM PBS (High challenge)	1.6×10^{10}	-	-	-
10 mM PBS + 0.01% Tween 20	2.0×10^7	-	600	-
10 mM PBS + 0.1% Poloxamer 188	2.1×10^7	-	590	-
10 mM PBS + 0.06% Triton X-100	2.4×10^7	-	485	570
10 mM PBS + 10% Sucrose	1.8×10^8	-	495	600
10 mM Phosphate buffer + 1M NaCl	1.2×10^8	-	485	650

The next series of experiments examined the effects of different excipients / additives on the bacterial challenge. Incubation of the *B. diminuta* in the different feed solutions had no effect on the viability of the bacteria (less than 10% reduction in CFU) with the single exception of Triton X 100 which caused an approximately 10-fold reduction in viability. The addition of the surfactants Tween 20, Poloxamer 188, and Triton X-100 had no effect on bacterial retention, with all permeate samples over the 20 min sterile filtration showing no detectable colonies (although this did correspond to a somewhat smaller log-reduction for the filtration in the presence of Triton X-100 due to the loss of viability in the presence of this surfactant). The effective size of the *B. diminuta* did seem to decrease in the presence of Triton X-100, sucrose, and 1 M NaCl (determined by NTA, see Table 1). However, this shift in size was not apparent when the *B. diminuta* were

treated with formalin (in the presence of these additives), confirming that this reduction in size was due almost entirely to a change in motility.

The Sartobran P was then challenged with a mixture of *B. diminuta* and the LAV at concentrations of 1×10^6 CFU / mL and 4.6×10^{10} LAV / mL, respectively. A sample of the feed after deposition on a 0.1 μm Isopore membrane is shown in the top panel of Figure 3. The bacteria (indicated by the red arrow) was approximately 0.64 μm in length and 0.35 μm in width, while the LAV particles are more spherical with diameters ranging from 0.15 – 0.42 μm , consistent with results from previous studies.¹⁵ In contrast, the permeate samples (bottom panel) showed no bacteria anywhere on the 0.1 μm Isopore membrane, despite every effort to examine the entire surface of the membrane. In contrast, the LAV were present at nearly the same concentration (and size) as in the feed. Thus, the Sartobran P provided a nearly perfect separation between the LAV and *B. diminuta*, despite the relatively small difference in size between these organisms.

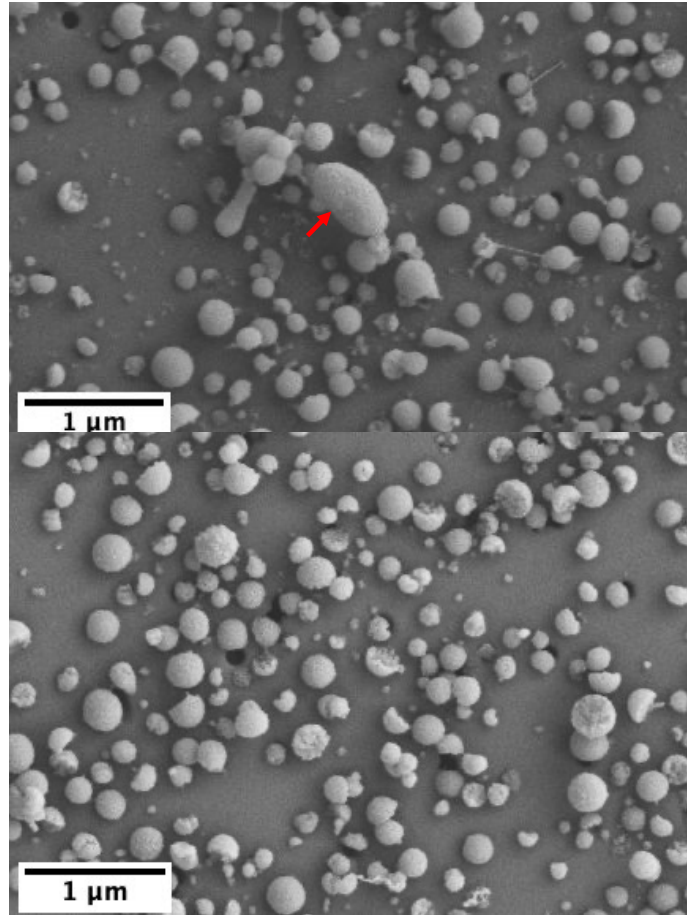


Figure 3: Scanning electron micrographs of the mixed feed of *B. diminuta* and LAV (top) filtered onto a 0.1 μm Isopore membrane with the red arrow indicating a single bacterium. Lower panel shows a sample of the permeate with no observable bacteria.

The pressure profiles and LAV transmission during sterile filtration experiments performed with the LAV alone and in a mixture of the LAV and *B. diminuta* at a constant filtrate flux of 300 L/m²/h (LMH) are shown in Figure 4. The addition of the *B. diminuta* had no effect on the LAV filtration. The transmembrane pressure increased nearly linearly with increasing volumetric throughput (cumulative filtrate volume divided by the membrane area). The fractional LAV transmission increased over the first 80 L/m², approaching a value of 0.8 by the end of the filtration experiment. The very low LAV concentration in the first few samples is due primarily to dilution

effects associated with the hold-up volume within the filter holder and membrane. The slower rise over the next $\approx 50 \text{ L/m}^2$ is due to saturation of a finite number of LAV binding / capture sites throughout the depth of the filter as discussed previously by Taylor et al.¹⁴

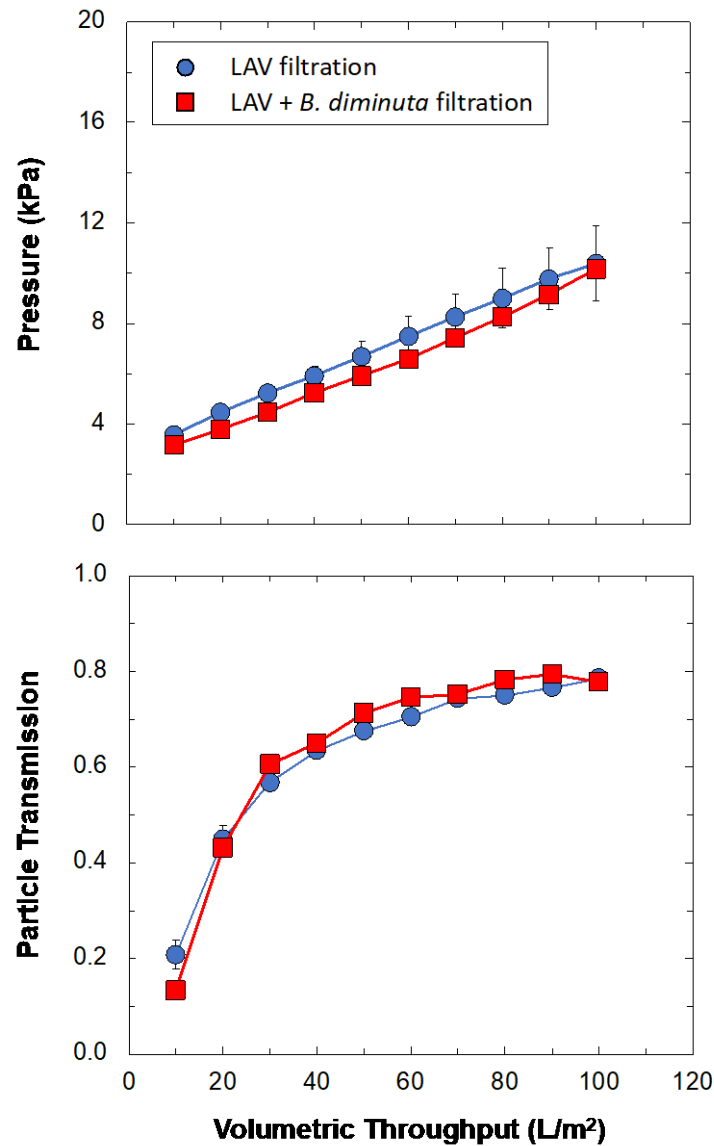


Figure 4: Transmembrane pressure (top panel) and LAV transmission (bottom panel) during sterile filtration of the LAV through $0.2 \mu\text{m}$ Sartobran P sterile filters both with and without added *B. diminuta*. Filtration performed at 300 LMH using a feed concentration of $4.6 \times 10^{10} \text{ LAV/mL}$.

Figure 5 shows the top surfaces of the 0.45 and 0.2 μm layers of the Sartobran P after 100 L/m^2 filtration of the feed containing the LAV and *B. diminuta*. The two layers were easily separated with a tweezer after soaking the filter in water. The 0.45 μm layer captured several *B. diminuta* in addition to a large number of LAV (possibly aggregates). A small number of *B. diminuta* were also captured at the entrance of the 0.2 μm layer. The size of these bacteria appears somewhat smaller than those captured in the 0.45 μm layer of the dual-layer Sartobran P.

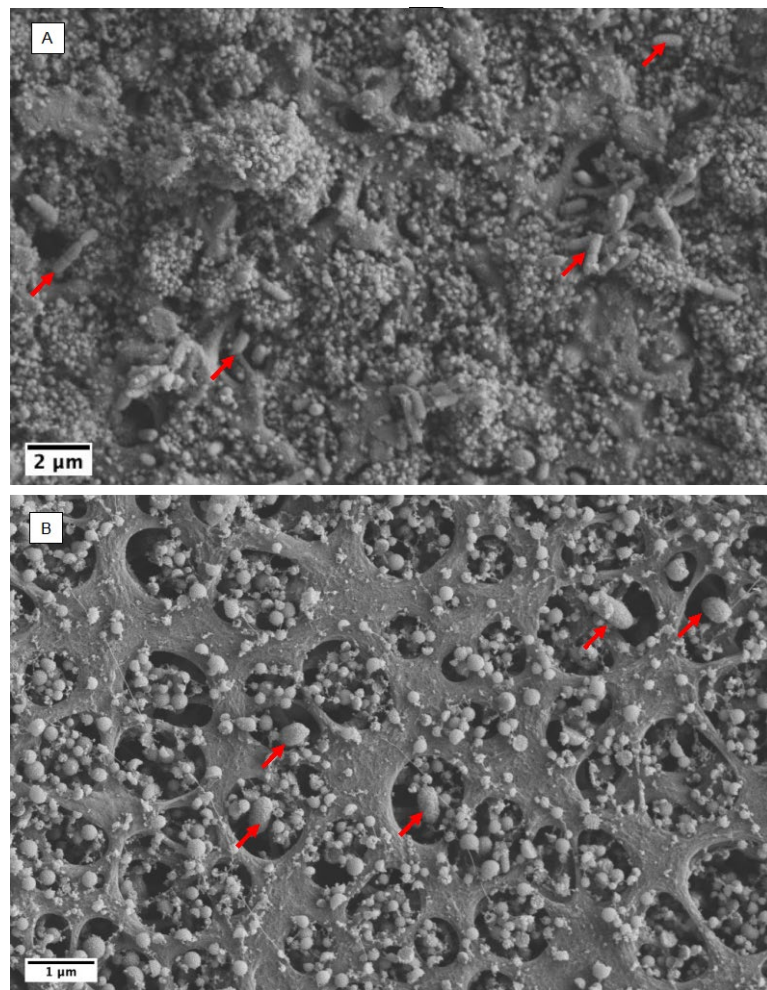


Figure 5: Scanning electron micrographs of the (A) 0.45 μm and (B) 0.2 μm layers of the Sartobran P after filtration of 100 L/m^2 of feed containing LAV and *B. diminuta*. The red arrows indicate the location of *B. diminuta*. Note the difference in scale bars between the two images.

Although some *B. diminuta* are clearly captured at the entrance to the pores in the individual layers of the Sartobran P, it is also possible that *B. diminuta* retention may occur at some of the same sites that capture the LAV. In order to explore this further, an experiment was performed in which the capture sites in the Sartobran P were first saturated by challenging the filter with LAV (with no bacteria) out to a volumetric throughput of 100 L/m². The feed was then immediately switched to a second reservoir containing the *B. diminuta* (no LAV) using a trivalve to eliminate any disruption in the flow. Permeate samples obtained throughout the *B. diminuta* challenge showed no colony forming units. Identical results were obtained using a Sartobran P filter that was first challenged with a suspension of more hydrophobic fluorescent nanoparticles (NP) in the presence of 0.01% Tween 20. These results clearly demonstrate that the Sartobran P retains its sterile filter capability even after saturation with LAV or NP.

The lack of bacteria breakthrough is likely due to the small, but meaningful, difference in size of the bacteria and LAV. Figure 6 shows the size distribution of the LAV and formalin-treated bacteria using DLS (top panel) and NTA (bottom panel); the results from DLS and NTA were statistically identical. The size determined after the formalin treatment is representative of the actual size of the *B. diminuta* since the formalin should eliminate the contribution from bacterial motility. The size distribution of the LAV in the feed and permeate were nearly identical, with a mean size of 260 nm as determined by DLS. The *B. diminuta* had a mean size of 570 nm with a range from about 300 nm to 1.5 μ m in diameter. The largest LAV particles are similar in size to the smallest bacterium. These larger LAV particles are likely retained by the Sartobran P consistent with the approximately 20% retention of LAV seen in the lower panel of Figure 4.

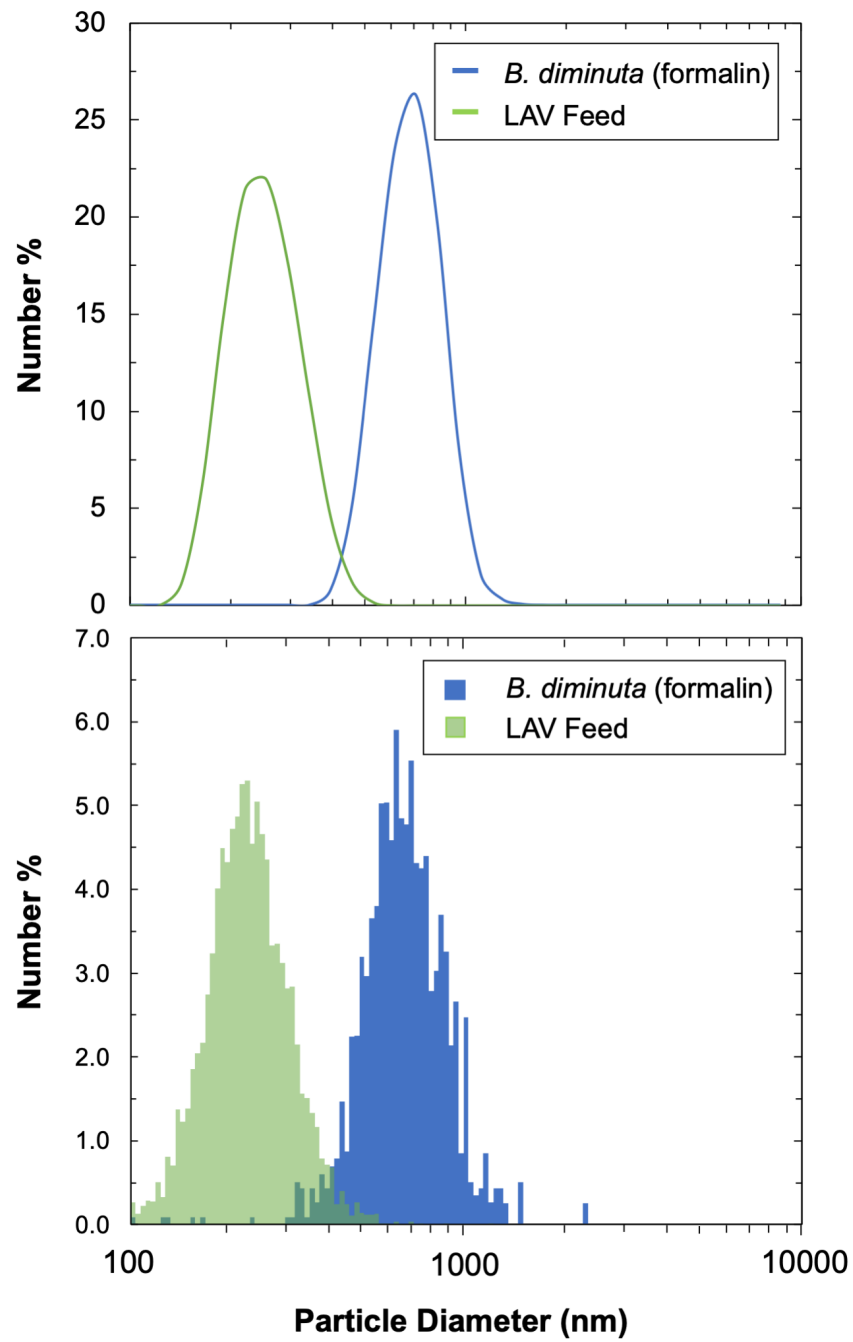


Figure 6: Size distribution for the LAV and the formalin-treated *B. diminuta* determined using DLS (top panel) and NTA (bottom panel).

4. Discussion

The results obtained in this study confirm that the dual-layer (0.45 / 0.2 μm) Sartobran P sterile filter, which shows high transmission of a 100 – 400 nm viral vaccine, completely retains *B. diminuta* (the ASTM standard organism for challenging sterile filters) under all investigated conditions. This was true even after challenging the Sartobran P with a suspension of LAV or polystyrene nanoparticles (which might have saturated potential capture sites) and in the presence of common additives that have been previously hypothesized as to increase the risk of *B. diminuta* breakthrough during sterile filtration (e.g., 10% sucrose or surfactants like Tween 20 or Triton X 100). *B. diminuta* capture was observed at the surface of both the 0.45 and 0.2 μm layers of the Sartobran P, with the 0.45 μm layer alone retaining about 90% of the bacteria in the challenge.

Although the Sartobran P showed complete bacterial retention under all conditions, the measured size of the *B. diminuta* was a function of the growth / solution environment. The *B. diminuta* were prepared according to ASTM F838-83, initially grown in a nutrient-rich TSB media in which the bacteria appeared rod-shaped with an average size of 0.4 x 1.2 μm as determined by SEM. Transfer of the *B. diminuta* to a nutrient-poor SLB media caused a reduction in size to about 0.3 x 0.6 μm in SEM images. These changes in size were confirmed using both DLS and NTA. Further reductions in size were observed upon addition of 10% sucrose and 0.06% Triton X 100, although in both cases this effect was due to an increase in motility (and thus the measured diffusivity) of the bacteria. This effect was eliminated by treating the bacteria with formalin, with the size of the killed bacteria unaffected by the different excipients. These results provide important insights into the retention of *B. diminuta* by the Sartobran P sterilizing grade filter under constant flux operation, confirming that this dual-layer filter can process a relatively large virus (with 80% recovery of the LAV) while still providing a sterile vaccine product.

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Conflict of Interest Statement

The authors do not have any conflicts of interest.

Author Contributions

Neil Taylor – Data curation, Formal analysis, Investigation, Writing – original draft.

Alex Wee – Data curation, Writing – review & editing.

Adam Kristopeit – Funding acquisition, Methodology, Resources, Writing – review & editing.

Wanli (Justin) Ma – Funding acquisition, Methodology, Resources, Writing – review & editing.

Sheng-ching Wang – Funding acquisition, Methodology, Resources, Writing – review & editing.

Andrew L. Zydney – _Conceptualization, Funding, Supervision, Writing – review & editing.

Data Availability Statement

The data and images presented in this paper are available from the corresponding author upon request.

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Figure Legends:

Figure 1: SEM images of *B. diminuta* in TSB (top) and SLB (bottom) deposited on 0.1 μm pore size Isopore membranes. The mean width and length of the bacteria grown in the TSB and SLB media were 0.4 x 1.2 μm and 0.3 x 0.6 μm , respectively.

Figure 2: Particle size distributions of the *B. diminuta* determined by DLS in both TSB and SLB growth media (without treatment by formalin).

Figure 3: Scanning electron micrographs of the mixed feed of *B. diminuta* and LAV (top) filtered onto a 0.1 μm Isopore membrane with the red arrow indicating a single bacterium. Lower panel shows a sample of the permeate with no observable bacteria.

Figure 4: Transmembrane pressure (top panel) and LAV transmission (bottom panel) during sterile filtration of the LAV through 0.2 μm Sartobran P sterile filters both with and without added *B. diminuta*. Filtration performed at 300 LMH using a feed concentration of 4.6×10^{10} LAV/mL.

Figure 5: Scanning electron micrographs of the (A) 0.45 μm and (B) 0.2 μm layers of the Sartobran P after filtration of 100 L/m² of feed containing LAV and *B. diminuta*. The red arrows indicate the location of *B. diminuta*. Note the difference in scale bars between the two images.

Figure 6: Size distribution for the LAV and the formalin-treated *B. diminuta* determined using DLS (top panel) and NTA (bottom panel).